Comparison of Real-time PCR fluorescent and non-fluorescent quenchers in standard amplification plots of delta-6 desaturase gene in PANC-1 cell line culture

M. Sahmani* M. Darabi** SH. Byagowi*** R. Najafipour****
T. Naserpour Farivar***** M. Darabi Amin******

*Assistant Professor of Clinical Biochemistry, Cellular and Molecular Research Center, Qazvin University of Medical Sciences, Qazvin, Iran
**Assistant Professor of Clinical Biochemistry, Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
***M.Sc. in Medical Biotechnology, Qazvin University of Medical Sciences, Qazvin, Iran
****Assistant Professor of Genetics, Cellular and Molecular Research Center University of Medical Sciences, Qazvin, Iran
*****Professor of Microbiology, Cellular and Molecular Research Center, Qazvin University of Medical Sciences, Qazvin, Iran
******Assistant Professor of Clinical Biochemistry, Maragheh Faculty of Medical Sciences, Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Abstract

Background: Employing non-fluorescent quenchers in Real-time PCR is appropriate for gene expression examination.

Objective: The objective of this study was to compare Real-time PCR fluorescent and non-fluorescent quenchers in standard amplification plots of delta-6 desaturase gene in PANC-1 cell line culture.

Methods: This analytical study was conducted in the Reference Laboratory affiliated to Qazvin University of Medical Sciences in 2012. Human pancreatic cancer cells (PANC-1) were cultured in 75 cm^2 flasks, $3x10^6$ cells were seeded in 6-well plates and were treated with specific intracellular signaling drugs. Changes in expression of delta-6 desaturase gene were examined with fluorescent and non-fluorescent quenchers using Real-time PCR in equal conditions, separately. Data were analyzed using student T-test and gene expression results were analyzed using $\Delta\Delta CT$ method with the assumption of 100% efficiency.

Findings: Employed quenchers showed different absorption of the fluorescence emitted by the reporter and caused different results in Real-time PCR. Using non-fluorescent quencher, the amplification plot was more precise and its baseline was lower. Therefore the signal to noise ratio (S/N) was decreased. Also, the Threshold cycle (Ct) value was lower because of increased Tm (melting temperature) and decreased non-specific bindings.

Conclusion: With regards to the results, non-fluorescent quencher is more appropriate compared to fluorescent quencher and can be a better alternative for current quenchers especially in allelic discrimination and SNP (single nucleotide polymorphism) studies.

Keywords: Real-Time PCR, Delta-6 Desaturase, Cell line

Coressponding Address: Maryam Darabi, Tabriz University of Medical Sciences, Drug Applied Research Center,Tabriz, Iran
Email: mdarabiamin@hotmail.com
Tel: +98-912-8803471
Received: 24 Apr 2013
Accepted: 11 Sep 2013